TRANSLATION NO. 2077

DATE: 29 November 1967

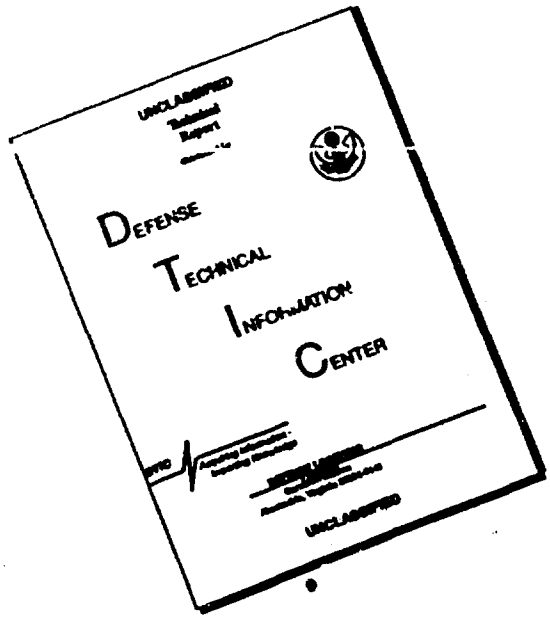
DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DDC.

This publication has been translated from the open literature and is available to the general public. Non-DOD agencies may purchase this publication from the Clearinghouse for Federal Scientific and Technical Information, U. S. Department of Commerce, Springfield, Va.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

UDC: 576.851.45-097.21

STUDY OF THE ANTIGENIC COMPOSITION OF SOME F. TULARENSIS STRAINS
REPORT II

COMPARATIVE ANTIGENIC COMPOSITION OF VIRULENT AND AVIRULENT BACTERIA

Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii
(Journal of Microbiology, Epidemiology and Immunobiology)
No 8, 1966, pp 104-108

V.V. Sukhar'

Determination of the antigenic structure of F. tularensis is important for immunological prophylaxis and diagnosis of tularemia. Nevertheless this problem has not been investigated enough to date.

Using serological examination methods a number of authors (El'bert and Gayskiy, 1941; Tinker, 1951; Olsuf'yev and Yemel'yanova, 1957) demonstrated that virulent F. tularensis have two antigens: a superficial and deep-seated one. They believe that avirulent bacteria contain only the latter.

Further studies (Ormsbee and Larson, 1955; Parnas et al, 1961; Dzhanpoladova and Semenova, 1962; Carlisle, Hinchliffe, 1962; Vosti, Ward and Tigertt, 1962) conducted with the use of immunochemical methods revealed a considerable number (up to eight) antigenic components, but failed to detect any distinctions related to virulence of tularemia strains.

In the present report are submitted the results of our comparative immunochemical study of the antigenic structure of virulent and avirulent strains of the pathogen of tularemia.

Virulent strain No 128 and avirulent strain No 21/400 of F. tularensis palaearctica were submitted to examination. The culture of strain No 128 was isolated from shrews in Krasnodarskiy Kray, and strain No 21/400 was obtained in the laboratory of the Institute of Epidemiology and Microbiology imeni Gamaleya, by attenuation of a virulent culture

isolated from a water rat*. In some experiments Gayskiy's vaccine strain No 15 (unreduced) was used for ancillary purposes.

Virulence of the strains was determined by the method of Olsuf'yev et al (1959). Immunogenicity of strains No 15 and 21/400 was tested by infecting mice that survived the experiment on determination of virulence. For this purpose 20 MLD of virulent strain No 128 were administered to each animal.

For mice, 1 MLD of strain No 128 consisted of 0.5 bacterial cells according to the standard of the State Control Institute imeni Tarasevich. Strain No 15 was immunogenic and had negligible residual virulence. Strain No 21/400 was avirulent and manifested no immunogenic properties when administered in a dosage of 100,000 bacterial cells.

A bacterial mass of all strains was obtained from 72-hour cultures incubated at 37° on glucose-cystine fish agar, killed for 18 hours with two volumes of acetone cooled to -30°, washed on a filter with acetone and desiccated under vacuum. It was established by electron microscopy that the killed bacterial cells were covered with a capsular substance and in this respect did not differ from live ones.

The antigenic composition of F. tularensis was studied by means of the reaction of double immunodiffusion in gel by the Ouchterloni method (quoted by Lebedev and Tsilinskiy, 1958). The antigen mixture to be examined consisted of a suspension of bacteria killed with acetone or solutions of corresponding water-saline extracts in a dosage of 10-20 mg [milligrams] per hole. The tularemic agglutinating horse serum used in this work in a titer of 1:1600 was prepared at the Odessa Institute of Epidemiology and Microbiology imeni Mechnikov. The water-salt extracts were obtained using a method described in our first report (Sukhar', 1965).

In order to determine the common antigenic features of the capsule and stroma of the virulent bacterium, a cell wall preparation was made ut of the bacterial mass of strain No 128. For this purpose 7.5 grams of bacterial mass was soaked in distilled water and after trituration with quartz sand for two hours, it was suspended in 150 ml [milliliters] of 2.5% NaCl solution. After 24 hours the suspension was centrifuged at 16,000 rpm (g=25,000 for one hour; as a result three layers were obtained: top (opalescent), middle (cloudy), and precipitate.

The middle layer was again centrifuged under the above conditions. Electron microscopically the centrifugate obtained consisted of a suspension of cell walls in a water-salt extract. The cell walls were precipitated at 25,000 g after preliminary addition to the centrifugate of

---

*The author expresses his deep appreciation to O.S. Yemel'yanova who supplied him with this strain.

middle layer of ammonia sulfate to 0.3 saturation. At the same centrifugation rate the precipitate was washed once with a $(NH_4)_2SO_4$ solution in the above-indicated concentration, and twice with distilled water. The preparation obtained met the cell wall requirements imposed by Shepart et al (1955): under an electron microscope it consisted of transparent fragments which were not precipitated at centrifugation at a rate of 16,000 rpm, the absorption spectrum showed no rise at a wave length of 260 millimicrons.

As a rule the suspension of acetone-killed bacterial cells of virulent strain No 128 (in a dosage of 15-20 mg) yielded 13 lines of precipitation with tularemic serum which we designated with letters of the Russian alphabet: A, B, V, G, D, Ye, Zh, Z, I, K, L, M, N. The same antigenic components with the exception of antigen A (Figure 1, on the insert between pages 112-113) were found in the cells of avirulent strain No 21/400. The antigenic composition of vaccine strain No 15 was analogous to that of the virulent strain. However, as demonstrated by the precipitation in gel reaction with titrated quantities of bacterial mass, it contained about 4-8 times less antigen A than the virulent strain. Thus, the vaccine strain occupied an intermediate position between virulent and avirulent strains with respect to this component.

A study of the antigen composition of water-salt extracts revealed that they were qualitatively identical to the water soluble antigen complexes of initial bacterial suspensions.

The cell wall suspension (in a dosage of 10-20 mg of dry preparation per hole)yielded one line of precipitation with tularemic serum identical with line A (Figure 2, insert between pages 112-113).

Thus, according to the gel precipitation reactions, the virulent strain of F. tularensis contains 12 antigenic components in common with the avirulent strain, and one additional A-antigen. The latter was present in all of the nine virulent strains we studied previously (Sukhar', 1965). Consequently A antigen is an additional specific antigen present in virulent strains, in contrast to avirulent strain No 21/400.

The studies of Tinker (1951), Olsuf'yev and Yemel'yanova (1957) revealed that the virulent F. tularensis contains thermostable and thermolabile antigenic complexes. In order to determine which antigenic components make up these complexes the gel precipitation reaction was conducted with autoclaving for an hour at 2 atmospheres of water-salt extracts of virulent and avirulent strains.

Tularemic serum produced lines A, V, Zh, Z, L, N with the autoclaved strain No 128 extracts (dosage: 15 mg of dry preparation per hole) and lines V, Zh, Z, L, N with strain No 21/400 extract. When small doses of autoclaved antigens (1-10 mg) were used only components A and N were demonstrable in the virulent strain and N in the avirulent.

The latter circumstance indicates that the bacterial cell contains considerable amounts of these components as compared to other thermostable antigens.

It must be noted that heating the water-salt extract of strain No 128 led to a considerable shift in line A in the direction of the serum hole. This is apparently due to haptenization of the antigen with heating.

Thus, the antigenic complex of virulent F. tularensis strain No 128 may be described as L (B, G, D, Ye, I, K, M) O (A, V, Zh, Z, L, N), where L is the complex of thermolabile antigens and O is the complex of thermostable antigens. The antigenic complex of avirulent F. tularensis strain No 21/400 is represented as L (B, G, D, Ye, I, K, M) O (V, Zh, Z, L, N).

The antigenic complex of vaccine strain No 15 is qualitatively identical with that of the virulent strain, however, for more precise expression a coefficient must be introduced to reflect the quantitative difference in antigen A content. The thermostable antigenic complex of the virulent strain (A, V, Zh, Z, L, N) is not equivalent to the thermostable (V, Zh, Z, L, N) or entire antigenic complex of the avirulent strain (B, V, G, D, Ye, Zh, Z, I, K, L, M, N).

According to the data of El'bert and Gayskiy (1941), the distinctive feature of the virulent culture is the large flocculated nature of agglutination with homologous antiserum. The avirulent culture produces only fine granular agglutinate. It would be natural to assume that the large flocculated agglutination is induced by the presence of A antigen in the cells. In order to solve this problem an experiment was performed with cross agglutination with absorbed sera. It was found that the tularemic serum, depleted with acetone-killed bacterial mass of the virulent strain does not agglutinate homologous or avirulent tularemic cultures and does not produce a precipitation line in gel. Serum depleted with avirulent strain antigens, on the contrary, into large flakes of virulent culture up to half of the initial titer and forms one precipitation line with a water-salt extract of strain No 128 (A antigen line). In other words, by means of absorption of tularemic serum by avirulent strain antgiens a monoreceptor A serum was obtained the antibodies of which caused the tularemia culture to amass in large flakes, when it contained the corresponding A antigen. Analogous results were obtained with the cell walls. The latter, according to the gel precipitation reaction, contained only A antigen and was agglutinated to the titer of both monoreceptor and nondepleted tularemic serum. The agglutinate was always in the form of large flakes.

Consequently the flocculated nature of the agglutinate, which is the distinctive feature of virulent tularemia strains is related to the presence of specific A antigen in the bacterial cells and of the corresponding A antibodies in the serum. High titers of this reaction indicate

superficial localization of A antigen in the bacterium. However, in contrast to the data of El'bert and Gayskiy (1941), Olsuf'yev and Yemel'yanova (1957) but in agreement with the conclusions of Tiner (1951) we found a specific antigen in the bacterial wall as well.

In the literature pertaining to the antigenic structure of F. tularensis a number of experimental data are submitted which contradicted the accepted characteristics of antigenic composition of this bacterium, but many of them are in complete agreement with the results of our experiments.

For example, Tinker (1951) indicates that the thermostable O antigen obtained from the virulent and vaccine strains yielded coarse dispersed agglutinate, while the O antigen isolated from the avirulent culture is made up of fine granules. On the basis of our data, the differences between the agglutinates must be attributed to the absence of thermostable A antigen in the avirulent strain.

Olsuf'yev and Yemel'yanova (1957) indicate that the antiserum against the avirulent bacterium, that was absorbed by autoclaved virulent culture, agglutinated live avirulent cultures to a titer of 1:640. This is indicative of a difference between the antigenic composition of autoclaved virulent and live avirulent culture, namely that the latter has a larger set of antigens. According to our data the correlation of antigens in the complexes being compared may be expressed as (A, V, Zh, Z, L, N):(V -- N). Obviously the autoclaved culture cannot induce depletion of all antibodies against the live avirulent culture, which is the reason for the aforementioned agglutination.

Thus, the submitted scheme of antigenic structure of the strains studied was found to be quite acceptable to explain the distinctions of serological reactions associated with tularemia. In view of the lack of marked cultural and serological differences between virulent strains of F. tularensis (Yemel'yanov, 1960, 1962), the scheme submitted may be considered common to all paleactic varieties of the bacterium; however further investigation is required to make definitive conclusions.

Conclusions

1. The presence of 12 antigenic components in avirulent strain No 21/400 of F. tularensis was demonstrated by the reaction of precipitation in gel.

2. Using the same method 12 antigenic components identical with those of the avirulent strain and an additional one, A antigen, were demonstrated in a virulent (No 128) and vaccine (No 15) strains.

3. A antigen is situated on the surface (in the capsule) and in the bacterial cell wall.

4. Large flake agglutination in the presence of tularemia is related to the presence of A antigen in the bacterial cells and of the corresponding A antibodies in the serum.

5. The virulent (No 128) and avirulent (No 21/400) strains have five thermostable and seven thermolabile antigeni components in common, while the virulent strain contains, in addition, thermostable A antigen.

BIBLIOGRAPHY

V.P. Dzhanpoladova, A.P. Semenova, Zh.mikrobiol.(Journal of Microbiology), No 4, 1952, p 27.

O.S. Yemel'yanova, in the book: Tulyaremiya (Tularemia), Moscow, 1960, p 51.

Idem, Zh. mikrobiol., No 4, 1962, p 3.

A.D. Lebedev, Ya.Ya. Tsilinskiy, Ibid, No 5, 1958, p 25.

N.G. Olsuf'yev, O.S. Yemel'yanova, Zh. gigi. epidemiol. (Prague) (Journal of Hygiene and Epidemiology), No 1, 1957, p 305.

N.G. Olsuf'yev, O.S. Yemel'yanova, T.N. Dunayeva, Vestn. AMN SSSR (Herald of the USSR Academy of Medical Sciences), No 6, 1959, p 51.

V.V. Sukhar', Zh. mikrobiol., No 11, 1965, p 38.

I.S. Tinker, Immunologicheskiye osnovy spetsificheskoy profilaktiki tulyaremii (Immunological Principles of Specific Tularemia Prophylaxis), Doctoral dissertation, Rostov-na-Donu, 1951.

B.Ya. El'bert, N.A. Gayskiy, Zh. mikrobiol., No 12, 1941, p 35.

H.N. Carlisle, V. Hinchliffe, S. Saslaw, J. Immunol.,Vol 89, 1962, p 638.

R.A. Ormsbee, C.L. Larson, Ibid, Vol 74, 1955, p 359.

J. Parnas, K. Burdzy, M. Cegielka, Bull. Acad. Pol. Sci., Ser. Sci. Biol., Vol 9, 1961, p 295.

S. Saslaw, H.N. Carlisle, V. Hinchliffe, Am. J. Med. Sci., Vol 244, 1962, p 175.

C.C. Shepard, E. Ribi, C.L. Larson, J. Immunol., Vol 75, 1955, p 7.

K.L. Vosti, M.K. Ward, W.D. Tigertt, J. Clin. Invest., Vol 41, 1962, p 1436.